CHROMATOGRAPHY EQUIPMENT CHARACTERIZATION

This application is a continuation of International Patent Application No. PCT/EP2011/073243, filed Dec. 19, 2011, which is hereby incorporated herein by reference in its entirety and which claims priority benefit to EP 10196288.4 filed Dec. 21, 2010.

The herein reported method is in the field of chromatography, especially in the field of preparative column chromatography. It is herein reported a method for the direct determination of the quality of the packing of a chromatography column based on in process data. With this method a saving in process time and resources can be achieved as an additional data acquisition solely for the purpose of column integrity determination can be eliminated.

BACKGROUND OF THE INVENTION

Today almost all polypeptides used in medicaments are prepared recombinantly. Due to strict regulatory guidelines and requirements, by-products have to be removed from the therapeutic polypeptide preparation as much as possible. Therefore, at least one chromatography step is employed in down stream processing of the bulk raw polypeptide after recombinant production. As the dimension of the chromatography equipment with respect to the yield of the fermentation process, especially the separation capacity of chromatography columns, is limited, a multitude of batches have to be processed in order to be able to provide the required amount of purified therapeutic polypeptide.

To ensure that each batch of the purified therapeutic polypeptide has the same pharmaceutical effect, a list of analytical parameters has to be fulfilled for each batch. This can only be achieved if the steps of the purification process operate consistently and efficiently. But, if one step of the purification process does not work properly the obtained product will most probably not pass the analytical tests and, in the worst case, this batch cannot be used. Therefore, it is necessary to provide methods for determining the performance and efficacy of purification steps.

Teeters, M. A. and Quinones-Garcia, I. (J. Chrom. A 1069 (2005) 53-64) report the evaluating and monitoring the packing behavior of process-scale chromatography columns by using the responses to conductivity-based pulse and step inputs derived from tracer experiments and in-process transitions, especially from measured residence time distributions. Norling, et al. (Norling, L., et al., J. Chrom. A 1069 (2005) 79-89) report the impact of multiple re-use of anion-exchange chromatography media on virus removal. The use of process data to assess chromatographic performance in production-scale protein purification columns is reported by Larson, et al. (Larson, T. M., et al., Biotechnol. Prog. 19 (2003) 485-492). Moscariello, J., et al., J. Chrom. A 908 (2001) 131-141 report the characterization of the performance of industrial-scale columns. The resolution and column efficiency in chromatography is reported by Vink, H., J. Chrom. 69 (1972) 237-242. Sarker, M. and Guiochon, G., J. Chrom. A 702 (1995) 27-44 report a study of the packing behavior of axial compression columns for preparative chromatography.

The use of an integrated form of the Gaussian distribution function allows for the description of the packed bed characteristics, whilst neglecting effects outside the packed bed itself, which potentially influence the evaluation (see e.g. PCT/EP2010/003813). The implementation of equipment characteristics in the evaluation of non-Gaussian distributions observed during the assessment of packed chromatographic beds has been described by Guiochon, G., et al. (FUNDAMENTALS OF PREPARATIVE AND NONLINEAR CHROMATOGRAPHY; Guiochon, G., et al. (eds), Elsevier Inc., San Diego (USA), 2nd edition (2006)).

SUMMARY OF THE INVENTION

With the method as reported herein a determination of the decrease in the separation efficacy and/or packing quality of a re-usable chromatography column packing can be determined without the need to use and inject a further tracer compound prior to the separation of the crude polypeptide solution for the determination of column material integrity or the need for historical data of this purification step.

The method reported herein allows for determination of packed matrix parameters separately and independently from contributions and effects of the equipment involved. This allows for the discrimination and/or allocation of separate physical effects and for a comprehensive characterization of the chromatography equipment used independent of the scale, such as in analytical scale and in industrial scale.

The first aspect as reported herein is a method for determining whether a re-usable chromatography column packing, which is used at least for the second time in a purification step of a multi-step purification process of a polypeptide, has reduced separation efficacy, e.g. compared to the separation efficacy when it was used for the first time in the same purification step of the same multi-step purification process of the same polypeptide, comprising the following steps:

a) identifying and determining the experimental data of an inert change of at least one physicochemical parameter of a mobile phase passing through said re-usable chromatography column packing,

b) determining the parameters of a function of formula I by fitting the experimental data of the inert change of the physicochemical parameter of the at least second use determined in step a),

c) determining the difference between the experimental data of the inert change of the physicochemical parameter of the at least second use determined in step a) and the function of formula I with the parameters determined in step b),

d) calculating the difference between the maximum value and the minimum value of the difference determined in step c) and normalizing said difference,

e) determining reduced separation efficacy of said re-usable chromatography column packing when the absolute value of the difference calculated in step d) is more than 0.1,

wherein the function of formula I is

$$yI = -\frac{1}{2}A \cdot \exp\left(-\frac{x}{t_0}\right) \left(\frac{\exp\left(\frac{2x_c t_0 + w^2}{2t_0^2}\right)\left(erf\left(\frac{\left(\frac{x-x_c}{w} - \frac{w}{t_0}\right)}{\sqrt{2}}\right)+1\right)}{\exp\left(\frac{x}{t_0}\right)\cdot erf\left(\frac{x-x_c}{\sqrt{2}\cdot w}\right)} \right) + y_0 \quad \text{formula 1}$$

with parameters A and y_0 describing the respective signal changes, x_c the mean value, and w the standard deviation of the underlying Gaussian distribution function, respectively, and parameter t_0 describing the time constant of the underlying exponential decay function, and